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Hydrogen Oxidation in Soils as a Possible Toxic-Effects Indicator

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Hydrogen Oxidation in Soils as a Possible Toxic-Effects Indicator[1]

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ABSTRACT

Efficient soil bioassays are needed in a screening array to determine the toxicities of industrial products and wastes. Hydrogen consumption is a common soil microbiological process that we evaluated as a possible soil indicator of toxic effects. Elemental tritium was used as a tracer to determine the H_2 oxidation rates in soils. The H_2 bioassay can be completed within 24 h using liquid scintillation counting of the tritium tracer. This test was used to evaluate the effects of known toxic chemicals (e.g., heavy metals, herbicides, and air pollutants), as well as a variety of suspected environmentally harmful compounds (e.g., waste waters, particulates, and sludges from industrial processes) on H_2 oxidation in soils. This bioassay responded to test compounds at concentrations shown to be toxic in other soil microbiological investigations.

Additional Index Words: **soil bioassay, tritium, H_2 oxidation, heavy metals, herbicides, NO_2, O_3, tricresyl phosphate; chemical process wastes.**

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Economical and efficient tests are needed to evaluate the toxicities of hazardous industrial products and waste materials. Often the hazardous materials are complex mixtures that make it even more difficult, or impossible, to classify their hazards by using the known physical and chemical properties. Thus, chemical and biological tests serve as better means to characterize toxicity levels. One approach uses a two-level environmental assessment; samples are screened simultaneously by a battery of chemical and biological tests (USEPA, 1979). The chemical tests are used to evaluate solubility, absorption, vapor pressure and octanol/water partitioning while the first-level biological tests (a series of short-term bioassays) are used to detect acute biological effects. Samples that warrant further evaluation will receive second-level testing. The bioassays may also individually serve as indicators of ecological perturbation.

Although some terrestrial ecological tests have been accepted for the level-1 program, there is a need to develop and evaluate other methods, especially soil bioassay procedures. Original recommendations included soil core microcosms, endogenous respiration, specific respiration, substrate degradation, N_2 fixation, nitrification, and SO_4 reduction (Waterland, 1979). These methods were evaluated on the basis of simplicity, re-

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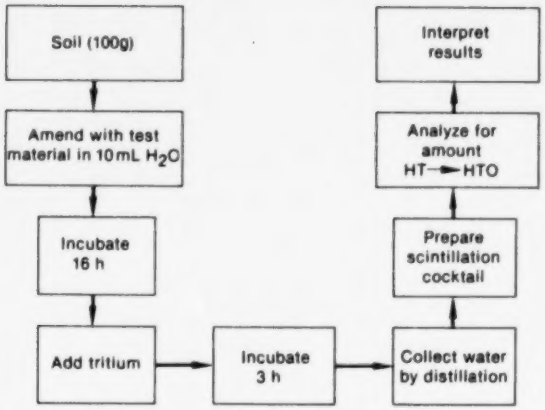

Fig. 1—Procedures for H_2 oxidation soil bioassay.

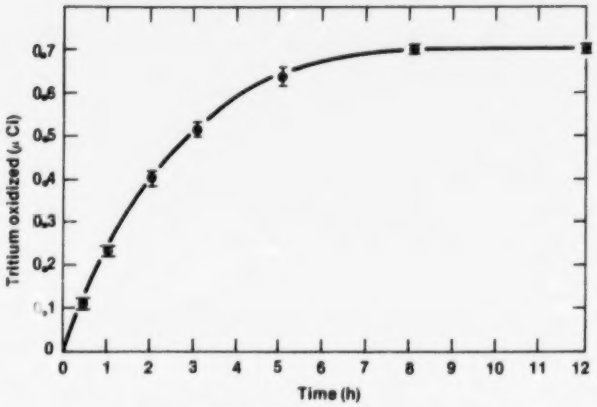

Fig. 2—Tritium oxidation in control soil.

producibility, cost, and time. Unfortunately, none of the current soil assays fulfill the required criteria.

A practical soil bioassay might utilize the consumption of H_2 by soil microorganisms. The process has been shown to occur in all soils tested to date (McFarlane et al., 1978, 1979; Seiler et al., 1977), and depends on one or more of the numerous facultative chemoautotrophic bacteria that can derive energy from the oxidation of H_2 (Repaske, 1966; Schlegel, 1966; Bongers, 1967; Ishaque et al., 1973; Bernard et al., 1974; Bradley et al., 1979). Organisms able to oxidize H_2 are found among the genera of *Pseudonomas, Alcaligenes, Corynebacterium,* and *Streptomyces* (Bradley et al., 1979); and *Hydrygenomonas, Sporovibrio,* and *Escherilbia coli* (Lamanni and Mallette, 1965). Collectively these organisms consume an estimated 10^{14} g H_2 each year (Conrad and Seiler, 1979).

While investigating the role of soil as a sink for tritium (McFarlane et al., 1978, 1979; McFarlane and Rogers, 1980; Bradley et al., 1979), we discovered that this process could be used as an effective bioassay. This report describes assays for the toxicity of gaseous, liquid, or solid compounds in pure or complex forms.

METHODS

The soil, a Calico series fine sandy loam (coarse over clayey, mixed (calcareous), thermic Aquic Xerofluvent) from southeastern Nevada (53.9% sand, 10.8% clay, 1.3% organic C; 12.7 meq/100 g CEC; pH 8.6), was air-dried, sieved to pass a 2-mm screen, and stored in plastic bags at room temperature (H_2 consumption was not altered after 1 year storage).

For the bioassay (carried out in triplicate; Fig. 1), 10 ml of water carrying the toxic compounds (i.e., liquids and sludge) or distilled water (i.e., when the toxicity of gaseous or particulate materials was being tested) was added to 100 g of the air-dried soil in a 1-L, round-bottom boiling flask (10 ml moisture/100 g soil was 25% of the soil moisture-holding capacity). The treated soils were incubated for 16 h in the dark at 25°C while being continuously perged with water-saturated air (1.0 L/min). The moisture loss was determined gravimetrically and when necessary make-up water was added.

To evaluate the activity of the treated soil, each flask was air-flushed for 30 s (withdrawing air from the center of the flask, at 20 L/min) and the flasks were then sealed with rubber stoppers. Immediately, 5 ml of N_2 containing 0.7 μCr of tritium (HT or T_2) was injected through the stopper. The initial atmosphere in the flask then contained approximately 0.5 ppm (V/V) of H_2 (Ehhalt et al., 1974) and a trace of HT. Since the consumption of the H_2/HT is rapid and begins immediately, care had to be used to charge the flasks sequentially; the same order was followed when the reactions were stopped. The charged flasks were returned to the environmental chamber (25°C) for 3 h.

The maximum rates of H_2 consumption occur when the H_2/HT concentration is highest, i.e., time zero (McFarlane et al., 1979). However, with this system, rate determinations could only be made accurately after measurable amounts of the product (HTO) were formed and the reaction interval was precisely measured. Repeated sampling of replicate treatments over time allowed the creation of a reaction curve (Fig. 2). This curve can be described by an exponential growth model:

$$Y = P_1 [1 - \exp(-P_2 t)] + E, \quad [1]$$

where:

Y = HT contents,
P_1 = the asymptotic HT content,
P_2 = the reaction rate parameter,
t = time in hours, and
E = the error function, assumed to be Gaussian.

By entering the asymptotic HT content (the amount injected) and the amount of HTO recovered at the time sampled, the rate parameter P_2 can be calculated. The derivative of the above formula yields the reaction rate:

$$\frac{dy}{dt} = P_1 P_2 \exp(-P_2 t). \quad [2]$$

Since $\exp(-P_2 t) = 1$ when $t = 0$, the maximum rate occurs at $t = 0$ and can be calculated by multiplying the amount of HT injected (P_1) and the determined rate parameter (P_2). Previous efforts focused on collecting the entire timed series (Bradley et al., 1979). However, additional study showed satisfactory results can be obtained for screening by selecting only one reaction time (in this case, 3 h).

To stop the measured reaction at the precise time (3 h), the labeled substrate (HT) was removed by flushing the flasks again with room air. The tritiated water was recovered from the soil by distillation (Fig. 3). Since high soil temperature leads to volatilization of organic matter, interfering with scintillation analysis, the following steps were used to avoid overheating. Ten milliliters of distilled water were added to each flask and distillation was stopped when 15 ml of HTO/H_2O had been collected. Although some isotopic fractionation of HTO/H_2O occurs during distillation (McFarlane, 1976), this was minimized by removing the same amount of water (15 ml) from each replicate. The flasks were covered with aluminum foil and the connection tube between the flask and moisture-receiving trap was wrapped with heating tape maintained at 150°C. A refrigerant (5°C) was circulated through the condenser. Liquid scintillation counting (the scintillation cocktail was made by mixing 1.5 g bis-MSB and 7.0 g PPO in 1-L P-xylene) was used to determine the HTO concentration, and the impact

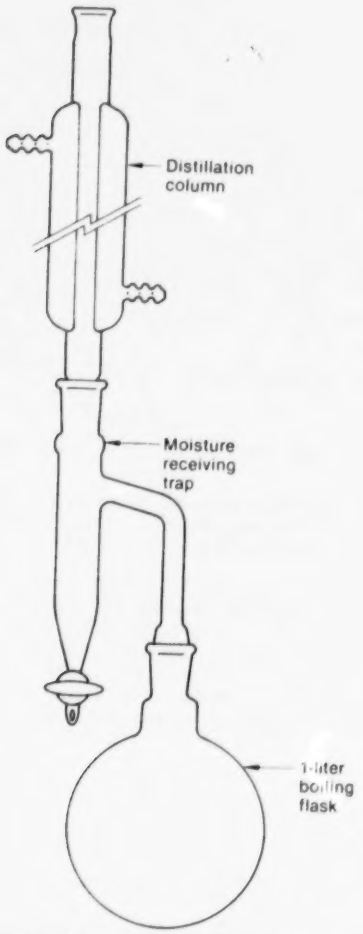

Fig. 3—Apparatus setup for collection of H_2O/HTO from soils exposed to tritium.

of the test compound was determined by comparing the calculated zero time rates in the treatments to those in the controls.

For gaseous pollutants, moist compressed air containing the toxic gas was used to purge the flasks (at a rate of 1 L/min) during the 16-h treatment period. Particulate pollutants were mixed with enough dry soil for three replicates (300 g). This amended soil was sealed in a plastic bag, the bag was filled with air, and the contents were hand-shaken for 5 min. The contaminated soils (100-g aliquots) were put into 1-L flasks, 10 ml of distilled water were added, and the test proceeded as usual. Control soils were prepared in the same manner except an inert substance (e.g., Ca-saturated montmorillonite clay powder) was added. Waste sludges were suspended in 10 ml of water and mixed thoroughly with the test soil. Organic liquids were first mixed with a Carrier (petroleum ether) and then added to the soil (300 g). The soil was mixed, and following the evaporation of the carrier 100-g aliquots of the soil were placed into the flasks and water was added. The control soils were likewise exposed to the carrier.

The experimental concentrations were selected on the basis of reported effects, when available. Consequently, various ranges were used. Initial experiments were intended to evaluate the impact of known toxic compounds. Success with these studies lead to testing complex environmental samples. For convenience in presentation, groupings were based on the type of pollutant and the concentration array used. Where possible, data were arranged to show plots of compound concentration vs. rate of H_2 oxidation, expressed as a percentage of the control.

The various groups and compounds are as follows: monomixtures of toxic compounds—(Cd as $CdCl_2$, Ag as $AgNO_3$, Hg as $Hg(NO_3)_2$, F

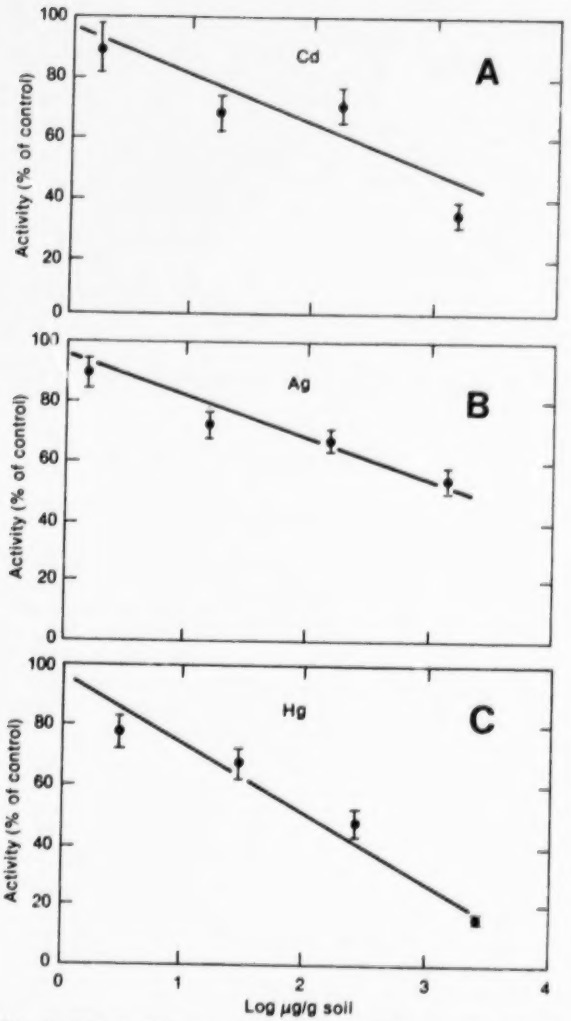

Fig. 4—Effects of heavy metals on H_2 oxidation (*A*) Cd, (*B*) Ag, (*C*) Hg.

as NaF, monuron, methanearsonic acid [MSMA], dalapon, 2,4-D + 2,4,5-T, O_3, NO_2, and tricresyl phosphate compounds); and complex environmental samples (process water from soil shale retort, cooling tower blowdown water from a steel plant, chemical process water, geothermal brine, particulates from Al and Cu smelter baghouses and a pressurized fluidized bed combustor, and process sludge from chemical and paint plants).

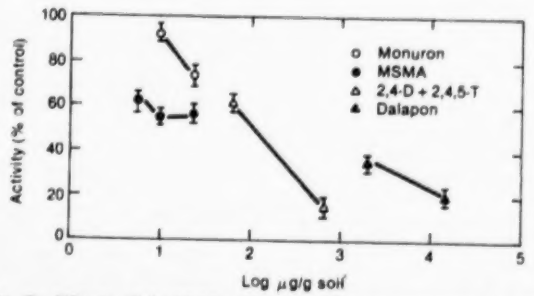

Fig. 5—Effects of herbicides on H_2 oxidation; monuron, MSMA, 2,4-D + 2,4,5-T, dalapon.

Table 1—Effect of industrial waste waters on H_2 oxidation in soil.

Material†	H_2 oxidation
	% of control rate ± S.D.
Oil shale retort process water no. 337	19.9 ± 1.0
Oil shale retort process water no. 153	3.5 ± 0.2
Cooling tower blowdown water from iron and steel plant	90.6 ± 1.0
Chemical process water	68.5 ± 3.0

† Concentration of sample as received.

RESULTS AND DISCUSSION

Monomixtures of Toxic Compounds

The toxic effects of heavy metals are presented in Fig. 4. The coefficients of determination (R^2) for Cd, Ag, and Hg were 0.85, 0.97, and 0.94, respectively, for a semilog regression analysis. From these high values, H_2 oxidation appears to be proportionally related to the log of the pollutant concentation. The difference in slopes suggests that the relative toxicity followed the order Hg > Cd > Ag. These metals have been shown to depress other soil microbial functions: 111 ppm Cd in soil decreased both microbial viability and ATPase activity;[3] 150 and 1,500 ppm Cd reduced soil respiration by 17 and 38%, respectively (Volk and Lighthart, 1977);[4] 54 ppm Ag depressed soil respiration (B. Lighthart, personal communication); and in clay and sandy soils, respectively, 100 ppm Hg reduced nitrogenase activity 70 and 90–100%, ammonification 15 and 24%, nitrification 49 ad 95%, and soil respiration 20% (Van Faassen, 1973).

Studies indicate that small amounts of F (2–10 ppm) stimulate CO_2 and NO_3 production in soils (Leroux, 1940), while concentrations of 84 ppm inhibited microbial activity in solution culture (Takami, 1969). Our studies have shown that F at 285 ppm reduced H_2 oxidation in test soil to 77% of the control.

The herbicides tested included: monuron, MSMA, dalapon, and a mixture of 2,4-D + 2,4,5-T; all decreased the H_2 oxidation rate (Fig. 5). Hydrogen oxidation was significantly depressed by MSMA even at the lowest concentration tested (5 μg/g soil). Grossbard (1976) reported that monuron at 500 ppm depressed soil respiration, and at 25–50 ppm in liquid cultures inhibited the nitrifying bacteria *Nitrobacter* spp.

Microbial responses to 2,4-D appear in the literature to be variable. For example, respiration was depressed at 100 ppm, denitrification was inhibited at 6 ppm, and urease activity was affected at 20 ppm (Grossbard, 1976). In contrast, 2,4-D stimulated the N_2-fixing organism *Clostridium pasteurianum*. With both concentrations we tested (78 and 780 ppm of 2,4-D + 2,4,5-T), H_2 oxidation was depressed.

Grossbard (1976) reported that 1.5–7.5 ppm dalapon inhibited soil bacteria and 60 ppm decreased respiration, while Greaves et al. (1976) reported no change in microbial populations with concentrations of 2,660

[3] L. Zibilske and G. H. Wagner. 1978. Growth of microorganisms in soil amended with sewage sludge containing three levels of Cd, Cr, or Ca. p. 148. *In* Agronomy Abstracts. Am. Soc. of Agron., Madison, Wis.

[4] B. G. Volk and B. Lighthart. 1977. Effects of metals on soil respiration in controlled environment microcosms. p. 39. *In* Agronomy Abstracts. Am. Soc. of Agron., Madison, Wis.

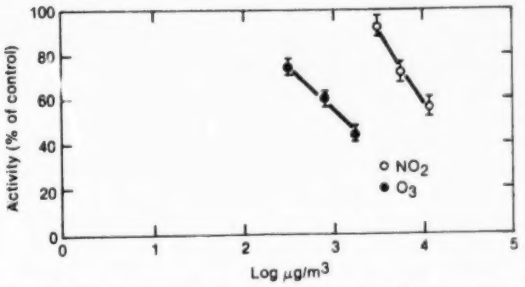

Fig. 6—Effects of gaseous air pollutants on H_2 oxidation: NO_2 and O_3.

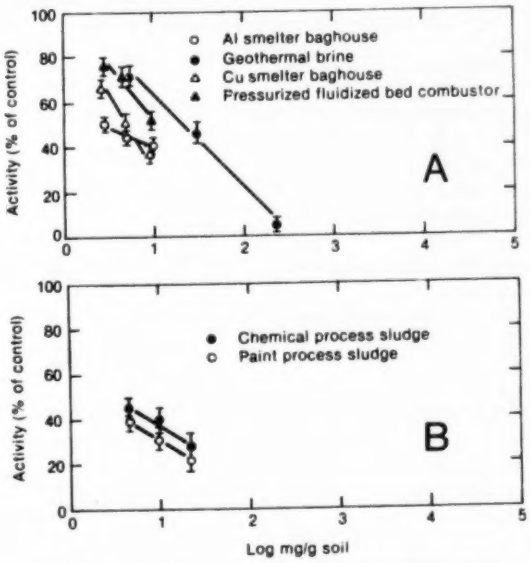

Fig. 7—Effects of environment waste stream effluents on hydrogen oxidation (*A*) geothermal brine, particulates from an Al smelter bag house, pressured fluidized bed combustor, and Cu smelter bag house; (*B*) chemical and paint-process sludge.

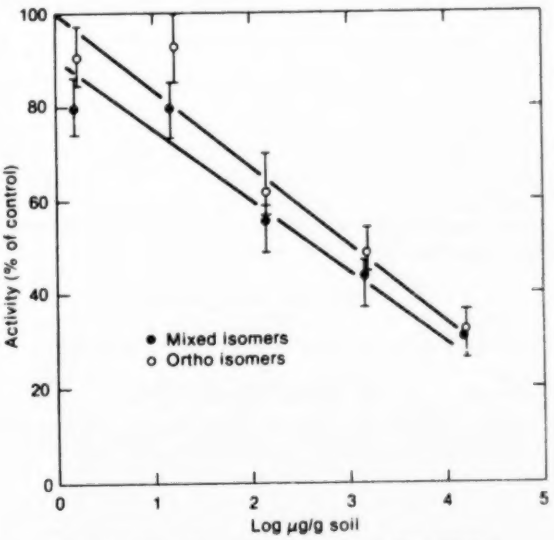

Fig. 8—Effect of tricresyl phosphates on H_2 oxidation.

ppm, and only a slight reduction in nitrification from applications ten times the normal field rates. Our data show a pronounced decrease in H_2 oxidation with high dalapon concentrations (2,000 μg/g soil).

The gaseous air pollutants studied were O_3 and NO_2. Grant et al. (1979) found that 20 mg/m³ NO_2 resulted in 13% reduction in glucose degradation. McCool et al. (1979) reported 1.8 mg/m³ O_3 reduced the amount of mycorrhizal infections. Our data show that both O_3 and NO_2 reduced H_2 consumption (Fig. 6) proportional to the concentrations, with O_3 being the more toxic fumigant.

Complex Environmental Samples

Except for the cooling tower blowdown water, undiluted complex environmental samples caused pronounced depression in the rate of H_2 oxidation (Table 1; Fig. 7A and 7B), especially for water from the oil shale retorting process. These data indicate that further biological and chemical testing of the complex environmental sample is justified.

Salt (NaCl) addition to soil caused a response similar to the geothermal brine (Fig. 7A). The osmotic stress imposed by the salt may also account for some of the other responses measured for the complex effluents. In addition, NaOH (100 μg/g soil) was added to soil to evaluate the change of pH observed when the oil shale water was tested. At the concentration used, this addition decreased the response to 80%. This solute amount was small compared with the salt addition, thus the decrease in activity is assigned to a change of soil pH to 9.4. (The same pH obtained with the addition of oil shale retort process water no. 153 in Table 1). The treatment response of the retort water in soil at the same pH was only 4%. Thus, a significant toxic response is implied.

Tricresyl phosphates are used in plasticized organic polymers hydraulic fluids, lubricants, and air conditioner filter media. Figure 8 shows the effects that various concentrations of the phosphates had on initial H_2 oxidation rates. The mixed isomers appeared to have little increased toxicity over the other form.

CONCLUSIONS

The hydrogen bioassay gave positive responses for test compounds (Fig. 4–8; Table 1) at concentrations shown to be toxic by other soil microbial tests. The H_2 bioassay is a simple, inexpensive, and sensitive test that can be completed within 24 h. This makes it an attractive candidate for level-1 screening.

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